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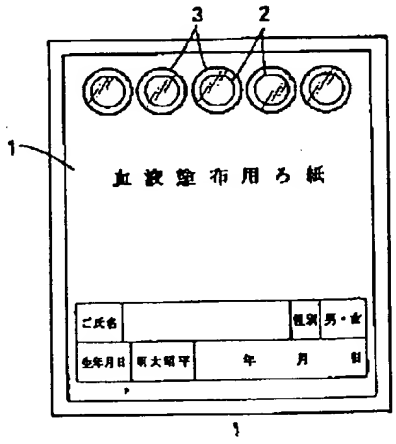
(54) BLOOD SPECIMEN COLLECTING CARD

(57) Abstract:

PROBLEM TO BE SOLVED: To provide a blood specimen collecting card by which the measured value as close as possible to the measured value of fresh blood can be obtained, and the measuring accuracy of serum-protein value, blood sugar value, prostate-specific acid phosphatase (PSA) value, hemoglobin Alc(Hb Alc) in which the measured values are affected by hemolysis, is improved.

SOLUTION: The card is made up of a porous film 2 with its pores of 1 to 3μm in diameter formed in the porosity rate of 4 to 20% on the surface of a card-like filter paper 1 impregnated with a glycolysis blocking material, and formed of hydrophilic resin made of, for example, polyethylene terephthalate or polycarbonate. A blood sample collecting card on which a porous film 3 coated or impregnated with a blood coagulation blocking material is lapped over to the bonded and fixed, is favorable. The examination of Hb Alc, etc., can be accurately performed by using only blood corpuscles adhered to the surface of the porous film 3, and since the blood corpuscles are not contained and few hemolysis occurs in the liquid-component absorbed by the card-like filter paper 1, the biochemical examination of blood serum value, blood sugar value, etc., which tend to be affected by hemolysis, can be exactly performed.



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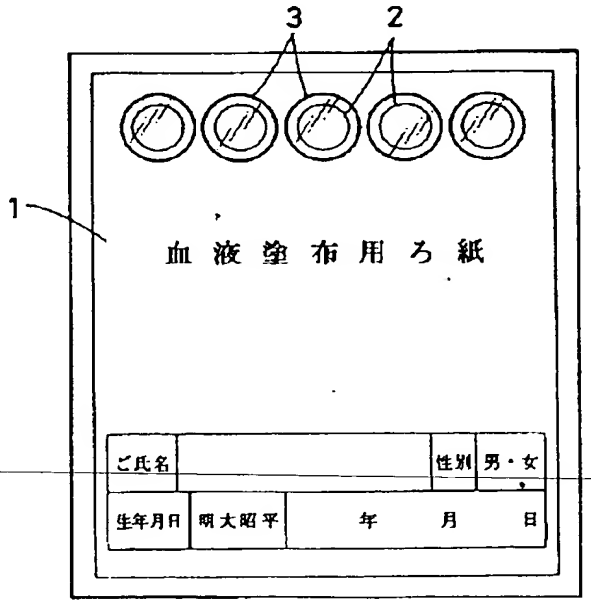
(54)【発明の名称】　血液検体採集カード

(57)【要約】

【課題】　新鮮血の測定値に可及的に近い測定値が得られ、特に溶血が測定値に影響する血清蛋白値、血糖値、前立腺特異酸フォスファターゼ（ＰＳＡ）値、ヘモグロビンＡｌｃ（ＨｂＡｌｃ）値などについて、測定精度が向上した血液検体採集カードを提供する。

【解決手段】　解糖阻止剤が含浸されているカード状濾紙１の表面に、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されている多孔性フィルム２からなり、例えばポリエチレンテレフタレートまたはポリカーボネートからなる親水性樹脂で形成され、好ましくは血液凝固阻止剤が塗布または含浸されている多孔性フィルム３を重ねて接着固定した血液検体採集カードとする。多孔性フィルム３の表面に付着した血球のみを用いてＨｂＡｌｃなどの検査が正確に行なえると共に、カード状濾紙１に吸収された液体成分には血球が含まれず溶血が少ないので、血清蛋白値、血糖値などの溶血の影響を受けやすい生化学検査を正確に行なえる。



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【特許請求の範囲】

【請求項１】　カード状濾紙の表面に、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されている多孔性フィルムを重ねて固定してなる血液検体採集カード。

【請求項２】　多孔性フィルムが、ポリエチレンテレフタレートまたはポリカーボネートからなる親水性樹脂で形成されている請求項１記載の血液検体採集カード。

【請求項３】　多孔性フィルムに、血液凝固阻止剤が塗布または含浸されている請求項１または２記載の血液検体採集カード。

【請求項４】　カード状濾紙に、解糖阻止剤が含浸されている請求項１～３のいずれか１項に記載の血液検体採集カード。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】この発明は、血液検査に供する血液検体を簡便に搬送または保存するための血液検体採集カードに関する。

【０００２】

【従来の技術】一般に、血液検査に使用する血液は、病院内で注射器で採取された血液を採用することが好ましいが、近年、在宅医療等のように病院外で採取された血液を検査することも要望されるようになり、自己採取した血液検体を病院または検査機関に送付（主として郵送）することによって、簡便に検診できる血液検体採集カードが開発されている。

【０００３】図１を利用して従来の血液検体採集カードを説明すると、このものはカード状の濾紙１の所定位置に間隔を開けて複数の血液滴下区分（円形）２を印刷し、その他の位置に必要事項を記入するようにした濾紙製のカードである。

【０００４】これを使用するには、被験者が自分の指頭を針状器具で刺して出血した血液を印刷された血液滴下区分２内にしみ込ませ、乾燥させた後、袋に密閉して郵送等により、医療検査機関へ搬送する。

【０００５】血液検体採集カードから血液検査を行なう際には、まず、カードの血液滴下区分内を所定の大きさに切り抜きまたは打ち抜き（パンチアウト）し、次いで生理的食塩水（０．７％食塩水）中に溶出させた血液を遠心分離し、血漿もしくは血清または血球についての各検査項目を常法により行なう。

【０００６】このような検査項目のうち、糖尿病患者の早期発見（スクリーニング）やその治療指導に利用される血糖値は、血漿中のブドウ糖量に対応する血糖値（以下、単に血糖値という。）と、赤血球の膜に結合した糖化ヘモグロビン量に対応するヘモグロビンＡｌｃ（以下、ＨｂＡｌｃと略記する。）との２種類が主である。

【０００７】このうちＨｂＡｌｃは、ヘモグロビンの主成分であるヘモグロビンＡのβ鎖のＮ末端アミノ酸残基のバリンに、グルコースがケトアミンの形で結合した糖

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化ヘモグロビンであり、赤血球の寿命中に分解されない安定した成分であるため、ＨｂＡｌｃの濃度が、現時点より１～２ヵ月以前の血糖値を反映する。

【０００８】このように血液検体採集カードからは、各種の血液検査を行なうことができ、これらの結果を病院で採血された検体から得られる結果と同様に評価できれば、在宅医療制度や成人病の診断に極めて有効である。

【０００９】

【発明が解決しようとする課題】しかし、従来の血液検体採集カードにしみ込ませた全血中の赤血球は、搬送する時に振盪等の機械的作用や浮遊液の浸透圧の低下等の物理的作用を受け、溶血し易い。

【００１０】そして、溶血により壊れた赤血球が通常の血液中の液体成分と混じると、それぞれの成分を遠心分離できなくなるので、赤血球または血漿もしくは血清をそれぞれ対象とする検査項目について、採血当初の測定値との誤差が大きくなり、正確な検査ができなくなるという問題点がある。

【００１１】このような測定値の誤差は、血清蛋白値、血糖値、前立腺特異酸フォスファターゼ（ＰＳＡ）値、ヘモグロビンＡｌｃ（ＨｂＡｌｃ）値などについて特に顕著に現われやすい。

【００１２】具体的には、血漿中に溶血した赤血球が混じると血糖値が過大または過少評価される場合があり、またＨｂＡｌｃの測定時に溶血した血液検体を使用すると、遠心分離によって分離される赤血球量が採血当初の量より少なくなり、いずれの場合も正確な測定ができない。

【００１３】そこで、この発明の課題は、上記した従来の問題点を解決して、血液検査における検査項目について、新鮮血を用いた測定値に可及的に近い測定値が得られる血液検体採集カードであり、特に溶血が測定値に影響する血清蛋白値、血糖値、前立腺特異酸フォスファターゼ（ＰＳＡ）値、ヘモグロビンＡｌｃ（ＨｂＡｌｃ）値などについて、測定精度が従来のものより一層に向上した血液検体採集カードを提供することである。

【００１４】

【課題を解決するための手段】上記の課題を解決するため、この発明においては、カード状濾紙の表面に、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されている多孔性フィルムを重ねて固定した血液検体採集カードとしたのである。

【００１５】または、前記多孔性フィルムに、血液凝固阻止剤が塗布または含浸されている血液検体採集カードを採用したのである。

【００１６】または、カード状濾紙に、解糖阻止剤が含浸されている上記の血液検体採集カードとしたのである。

【００１７】この発明の血液検体採集カードは、所定の孔径および所定の多孔率の多孔性フィルムにフィルター

の作用があり、カードに滴下された血液中の成分のうち、血小板や白血球および赤血球を除く液体成分のみが多孔性フィルムを通過して濾紙に移行吸収され、分離された赤血球などの血球は、多孔性フィルムの表面に付着する。

【００１８】上記の血液検体採集カードにおける多孔性フィルムに対し、血液凝固阻止剤を塗布または含浸したものは、血液成分のうち血清およびフィブリノーゲンが多孔性フィルムを通過するので、濾紙中に血漿が浸透する。また、多孔性フィルムに血液凝固阻止剤が付着していない血液検体採集カードでは、フィブリンが除去された血清が濾紙中に浸透する。

【００１９】このようにして多孔性フィルム上で血球を分離し、他の液体成分（血清または血漿）を濾紙に浸透させてそのまま乾燥させると、多孔性フィルムに付着した血球を用いてＨｂＡｌｃなどの検査が正確に行なえると共に、濾紙に吸収された溶血の影響の少ない血清または血漿を用いて、血清蛋白値、血糖値などの生化学検査を正確に行なえる。

【００２０】カード状濾紙に、解糖阻止剤が含浸された血液検体採集カードは、濾紙にしみ込んだ血清または血漿中の糖が解糖し難い。そのため、多孔性フィルム上に糖が移行しないので、多孔性フィルムに付着した血球を用いていっそう正確に血糖値を測定できる。

【００２１】

【発明の実施の形態】この発明に用いるカード状濾紙は、特にその厚さや形状を限定したものではなく、血液に不活性で均一な多孔性を示す周知の濾紙、特には血液採集用濾紙を採用できる。このようなカード状濾紙は、たとえばベルギー国ワットマン社製のＢＦＣ１８０（商品名）などの市販品を採用できる。

【００２２】因みに、ワットマン社製のカード状濾紙の製造方法は、特開昭５２－１１７９２１号公報に記載されているように、シリコーン樹脂などの結合剤を添加した水性媒体中にアルミナファイバーやジルコニアファイバーなどの繊維（繊維径０．００１～１０μｍ）を分散させ、これを所定のｐＨ（ｐＨ２．８～３．５程度）でマット状に抄造時に前記結合剤を析出させ、その後、前記マットを圧搾して均質な多孔性濾紙を得る方法である。

【００２３】図１に示すように、このようなカード状濾紙１には、間隔を開けて直径１～２ｃｍの円形などからなる複数の血液滴下区分２を印刷する。時間または条件（食前、食後等）の別による血液成分の変動を調べるには、血液滴下区分２は複数箇所に設け、順序を決めて使用することが好ましい。

【００２４】カード状濾紙１に含浸する解糖阻止剤としては、多孔性フィルム上に糖が移行することを防止できるものであれば、特に限定することなく周知のものを採用できるが、特にフッ化ナトリウム（ＮａＦ）を採用して好ましい結果を得ている。

【００２５】また、この発明に用いる多孔性フィルムは、７～２５μｍ程度の厚さの合成樹脂製フィルムであって、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されているものを採用できる。このような合成樹脂は、血液を常圧で浸透させ得るように、親水性の良い樹脂を採用することが好ましく、例えばポリエチレンテレフタレート（以下、ＰＥＴと略記する。）またはポリカーボネート（以下、ＰＣと略記する。）を採用して好ましい結果を得ている。

【００２６】多孔性フィルムは、前記所定範囲未満の孔径または多孔率のものでは、血液の液体成分（主として血清または血漿）がこのフィルムを通過し難くなって好ましくなく、また、所定範囲を越える過大な孔径または多孔率のものでは、赤血球などの血球を分離できず好ましくない。

【００２７】なお、前記孔径または多孔率から孔密度を計算によって求めると、10^5～6×10^8（pores/cm^2）であり、上記した条件を満足する市販のＰＥＴまたはＰＣからなる多孔性フィルムとしては、ワットマン社製：サイクロポア（商品名）などを採用することができる。

【００２８】また、多孔性フィルムに塗布または含浸する血液凝固阻止剤としては、フィルム上でフィブリノーゲンなどの血液凝固因子の作用を阻害するヘパリンなどの周知の血液凝固阻止剤を採用できる。ヘパリンナトリウムを塗布または含浸する場合には、多孔性フィルムの多孔性（孔径、多孔率）を変化させないように、蒸留水で希釈して５～１０単位（ＩＵ）／ｍｌ濃度としたヘパリン溶液に多孔性フィルムを含浸し、室温下で乾燥させるか、またはスプレーなどによってフィルム表面に塗布する方法を適宜に採用できる。

【００２９】そして、図１および図２に示すように、上記した多孔性フィルム３は、前記したカード状濾紙１の表面に示された血液滴下区分２を個別に覆うか、または図３に示すように、複数の血液滴下区分２をまとめて覆う大きさの方形状または多角形状、円形その他の周知形状に裁断し、これをカード状濾紙３の血液滴下区分２の外側に固定する。固定手段は、通常、接着剤を使用することが簡便であり、図２に示すように、例えばシリコーン樹脂系接着剤のような所要の生化学検査に支障がない接着剤４を塗布して用いる。

【００３０】血液検体採集カードに血液を採取した後、これを検査機関等に郵送等する場合には、気密性、遮光性および断熱性の良い容器に入れておくことが好ましい。多数の血液検体採集カードをまとめて送付する場合には、断熱性のよい発泡スチロールなどからなる密閉容器を利用できるが、個別に郵送する場合には、アルミニウム箔をラミネートしたシール付きの封筒を利用することが好ましい。

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【００３１】

【実施例】

〔実施例１〕まず、カード状濾紙としてワットマン社製濾紙（ＢＦＣ１８０）を採用し、図１および図２に示すように、縦１００ｍｍ、横９６ｍｍの方形状カード状濾紙１を形成し、その上縁に沿って一列に血液滴下区分２として内縁８ｍｍ、外円１０ｍｍの二重円を５個と検査に必要事項を黒色インクにて印刷した。

【００３２】次いで、ワットマン社製の多孔性フィルム３である親水性ポリカーボネート製サイクロポア（厚さ１０～２５μｍ、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されているもの）を直径１０ｍｍの円形に裁断し、これを前記二重円の間に塗布したシリコーン樹脂系接着剤で前記二重円を覆うように接着固定した。

【００３３】血液検査を行なうときには、被験者の手の指先をランセットで突き刺し、出血した血液を多孔性フイルム３に滴下し、その下層のカード状濾紙１に血漿や血清が移行するのを確認した後、これを充分に乾燥し、アルミニウム箔のラミネート紙からなる封筒（アルミパック）に入れて内部の空気を押し出し、クリップで密封した。血液検体は、３人の被験者から下記検査項目①～④用に、それぞれ１０回ずつ採取した。

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＊【００３４】（溶血が検査測定に影響を及ぼす検査項目）

① 血清蛋白（ＴＰ）の定量：ビュレット法（臨床病理，19:427,1971）により行ない結果を表１に示した。

② 血糖（ＢＳ）の定量：グルコースオキシダーゼ法（臨床検査法提要 P440～441 、金井泉）により行ない結果を表２に示した。

③ 前立腺特異酸フォスファターゼ（ＰＳＡ）の定量：（The Journal of Urology Vol.151 1283 ～1290 May 1994.USA）により行ない結果を表３に示した。（多孔性フィルタを用いた検査項目）

④ ヘモグロビンＡｌＣ（ＨｂＡｌｃ）の定量：エンザイムイムノアッセイ（日本臨床検査自動化学会会誌1993 JJCLA Vol 18, No6 P754 ～758）により行ない結果を表４に示した。

【００３５】〔比較例１〕カード状濾紙としてＳＲＬ社製ラピディアオートＨｂＡｌｃ（商品名）を採用し、濾紙の上を多孔性フィルタで覆わないこと以外は実施例１と全く同様にして上記①～④の検査を行ない、その結果を表１～４中に併記した。

【００３６】

【表１】

血清蛋白（ＴＰ）の定量

検体・番号 / 採血回数と単位		検体 A		検体 B		検体 C	
		実施例１	比較例１	実施例１	比較例１	実施例１	比較例１
1	g/dl	8.4	7.9	4.2	3.7	2.0	1.8
2	g/dl	8.4	7.8	4.4	3.7	1.9	1.7
3	g/dl	8.3	8.0	4.3	3.8	1.9	1.7
4	g/dl	8.4	8.0	4.3	3.8	1.9	1.7
5	g/dl	8.5	8.3	4.4	3.8	2.2	1.7
6	g/dl	8.1	8.0	4.4	3.8	2.1	1.6
7	g/dl	8.3	7.9	4.3	3.8	1.7	1.5
8	g/dl	8.4	7.7	4.3	3.9	1.9	1.9
9	g/dl	8.3	7.8	4.3	3.8	2.2	1.7
10	g/dl	8.3	7.8	4.4	3.8	2.3	1.7
平均値	g/dl	8.34	7.92	4.33	3.79	2.01	1.7
変動係数（%）		1.2	2.1	1.6	1.5	9.2	6.2

【００３７】

【表２】

血糖（ＢＳ）の定量

検体・号		検体 A		検体 B		検体 C	
採血回数と単位		実施例１	比較例１	実施例１	比較例１	実施例１	比較例１
1	mg/dl	502	482	154	138	78	68
2	mg/dl	498	486	156	148	78	64
3	mg/dl	508	462	154	144	76	68
4	mg/dl	484	478	150	140	78	68
5	mg/dl	504	480	148	148	68	68
6	mg/dl	492	468	152	140	72	64
7	mg/dl	482	482	152	140	78	68
8	mg/dl	498	480	152	142	78	66
9	mg/dl	488	484	150	144	78	64
平均値	mg/dl	495.2	478.2	152.2	142.6	76.0	66.6
変動係数（%）		1.7	1.6	1.6	2.3	4.5	2.8

【００３８】　　＊　＊【表３】

前立腺特異酸フォスファターゼ（ＰＳＡ）の定量

検体・番号		検体 A		検体 B		検体 C	
採血回数と単位		実施例１	比較例１	実施例１	比較例１	実施例１	比較例１
1	ng/ml	21.8	19.4	7.2	8.4	1.8	2.4
2	ng/ml	17.6	20.4	8.4	9.6	1.6	2.2
3	ng/ml	18.3	22.6	8.8	9.0	1.6	1.8
4	ng/ml	18.8	18.6	9.4	9.6	1.6	2.6
5	ng/ml	20.6	19.2	8.2	9.8	1.8	2.4
6	ng/ml	22.4	24.4	8.3	9.2	2.2	2.2
7	ng/ml	19.8	18.4	8.8	9.2	1.8	2.6
8	ng/ml	19.2	19.8	9.4	9.6	1.8	2.2
9	ng/ml	19.2	19.8	9.0	9.8	1.6	2.6
１０	ng/ml	18.4	18.2	9.4	9.2	1.8	2.2
平均値	ng/ml	19.61	20.06	8.69	9.34	1.76	2.32
標準偏差（%）		7.8	9.6	8.0	4.3	10.4	10.9

【００３９】　　【表４】

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ヘモグロビンＡｌＣ（ＨｂＡｌｃ）の定量

検体・番号 / 採血回数と単位		検体Ａ		検体Ｂ		検体Ｃ	
		実施例１	比較例１	実施例１	比較例１	実施例１	比較例１
１	%	10.4	9.6	3.2	2.4	7.8	6.8
２	%	9.8	9.6	8.4	2.8	7.8	6.4
３	%	11.6	9.2	3.4	3.0	7.6	6.8
４	%	9.6	8.4	3.0	2.6	7.8	6.8
５	%	10.8	9.6	2.8	2.6	6.8	6.8
６	%	9.8	9.2	3.0	2.8	7.2	6.4
７	%	9.6	9.6	3.0	3.0	7.8	6.8
８	%	9.6	9.6	3.4	3.0	7.8	6.6
９	%	9.8	9.6	3.2	2.4	7.6	6.8
１０	%	9.6	9.6	3.2	2.6	7.8	6.4
平均値	%	10.06	9.4	9.14	2.7	7.6	6.7
標準偏差（%）		6.7	4.1	6.0	8.7	4.5	2.8

【００４０】表１～４（検査①～④）の結果の回帰式と相関係数は、以下の通りである。

①　ＴＰの測定：回帰式Ｙ＝０．９８Ｘ－０．３６　相関係数ｒ＝０．９９

②　ＢＳの測定：回帰式Ｙ＝０．９８Ｘ－７．３７　相関係数ｒ＝０．９９

③　PSA の測定：回帰式Ｙ＝０．９９Ｘ＋０．６　相関係数ｒ＝０．９９

④　ＨｂＡｌｃ：回帰式Ｙ＝０．９６Ｘ－０．３９　相関係数ｒ＝０．９９

表１～４および上記の結果から、検査項目①～④の変動＊係数は各項目ともにほぼ５％以下であり、サンプリングによる影響は認められなかった。回帰式より検査①のＴＰおよび検査④のＨｂＡｌｃは２～３％低い結果であったが、相関係数は両者とも０．９９と良好であった。

【００４１】また、実施例１の血液検体採集カードに対しては、血液採取後に密封状態のまま１０日間常温で保存し、ＨｂＡｌｃ値の経日変化を調べ、結果を表５中に示した。

【００４２】

【表５】

ＨｂＡｌｃ値の経日変化

検体 / 期間：日		1	2	3	4	5	6	7	8	7	8
検体Ａ	Ａ-1	2.2	2.1	2.4	2.4	2.2	2.1	2.0	2.2	2.2	2.1
	Ａ-2	2.2	2.1	2.4	2.2	2.2	2.1	2.1	2.1	2.0	2.0
	（平均）	2.2	2.1	2.4	2.3	2.2	2.1	2.1	2.1	2.1	2.1
検体Ｂ	Ｂ-1	5.2	5.1	5.1	5.1	5.2	5.2	5.1	5.0	5.0	5.0
	Ｂ-2	5.1	5.2	5.1	5.1	5.2	5.2	5.1	5.1	5.1	5.1
	（平均）	5.2	5.1	5.1	5.1	5.2	5.2	5.1	5.1	5.0	5.1
検体Ｃ	Ｃ-1	7.1	7.2	7.2	7.2	7.2	7.0	7.0	7.0	7.0	6.9
	Ｃ-2	7.1	7.0	7.1	7.1	7.1	7.0	7.0	7.0	6.9	6.9
	（平均）	7.1	7.1	7.2	7.2	7.2	7.0	7.0	7.0	7.0	6.9

【００４３】検体Ａ、Ｂ、ＣそれぞれのＨｂＡｌｃ値の平均値、標準偏差、変動係数を下記にまとめて示した。

検体Ａ：平均値２．１７、標準偏差０．１１、変動係数４．９％

検体Ｂ：平均値５．０８、標準偏差０．１６、変動係数３．２％

検体Ｃ：平均値７．０５、標準偏差０．１８、変動係数２．５％

このように検体Ａ、Ｂ、Ｃの１０日間の変動係数は、５％以下で良好であり、実施例１の血液検体採集カードが、１０日間の常温保存に耐えることがわかった。なお、検体Ａ、Ｂ、Ｃの検査に用いたアルミパック内の大腸菌およびブドウ状球菌の有無を期間初日と５日後、および１０日後に調べたが、これらの菌はいずれも検出されなかった。

【００４４】〔実施例２〕実施例１において、カード状濾紙の円形の血液滴下区分内に解糖阻止剤であるフッ化ナトリウムを１ｍｇ／ｍｌになるようにしみ込ませたこ

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と以外は全く同様にして、血液検体採集カードを作成した。血液滴下区分内（内径８ｍｍの円内）の一面にフッ化ナトリウムをしみ込むませるには、５０μｌ（フッ化ソーダ濃度２０ｍｇ／ｍｌ）が最適である。

【００４５】実施例２については、１０人の被験者からフッ化ナトリウム入り採血管（テルモシリンジ２１Ｇ）で静脈血を採取し、実施例２の血液検体採集カードに５０μｌ滴下し、血糖値（ＢＳ）を前記検査方法②によって調べ、この結果を表６に示した。

【００４６】なお、濾紙にフッ化ナトリウムを含浸しない実施例１についても上記同様の被験者から採血し、測定したＢＳを表６中に併記した。また、血液検体採集カードを使用しない従来法として、採血管内の血液をそのまま遠心分離して得た血清から血糖（ＢＳ）値を測定し、結果を表６中に併記した。

【００４７】

【表６】

検体・番号 / 採血回数と単位		検体Ａ	検体Ｂ	検体Ｃ
		採血管	実施例１	実施例２
1	mg/dl	96	83	94
2	mg/dl	78	61	84
3	mg/dl	102	82	98
4	mg/dl	154	136	148
5	mg/dl	218	186	203
6	mg/dl	98	94	102
7	mg/dl	90	84	96
8	mg/dl	74	68	76
9	mg/dl	46	48	54
１０	mg/dl	122	102	118
平均値	mg/dl	107.8	94.4	107.3

【００４８】Ａ（Ｙ）：Ｂ（Ｘ）の回帰式は、Ｙ＝１．１８Ｘ－４．３であり、従来法と比較してＢは１０％強低い値であり、相関係数は０．９９と良好であった。

【００４９】Ａ（Ｙ）：Ｃ（Ｘ）の回帰式は、Ｙ＝１．１５Ｘ－１５．７であり、Ｂと比較して血糖値の減少、＊すなわち解糖の昂進はフッ化ナトリウムを濾紙にしみ込ませる手段によって改善されたことがわかる。

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【００５０】なお、上記実施例では、検査項目①～④に示す各試験方法によってＴＰ、ＢＳ、ＰＳＡ、ＨｂＡｌｃを測定したが、胃癌のマーカーであるペプシノゲン抗体をI^{125}ラベルすることによってγーカウンターで測定したりすることもでき、またＨｂＡｌｃ値などは、分光光度計を用いて測定する方法、その他の常法を採用してもよいのは勿論である。

【００５１】

【発明の効果】この発明は、以上説明したように、カード状濾紙の表面に、孔径１～３μｍの細孔が多孔率４～２０％の割合で形成されている多孔性フィルムを重ねて固定した血液検体採集カードとしたので、新鮮血の測定値に可及的に近い測定値が得られる血液検体採集カードであり、特に溶血が測定値に影響する血清蛋白値、血糖値、前立腺特異酸フォスファターゼ（ＰＳＡ）値、ヘモグロビンＡｌｃ（ＨｂＡｌｃ）値などについて、測定精度が向上した血液検体採集カードになるという利点がある。

【００５２】また、前記多孔性フィルムに対して血液凝固阻止剤を塗布または含浸した血液検体採集カードは、濾紙中に血漿が吸収され、血漿を検体とする生化学的検査に有用である。

【００５３】また、カード状濾紙に、解糖阻止剤が含浸されているものでは、濾紙に一旦しみ込んだ血清または血漿中の糖が解糖せず、多孔性フィルム上に移行することが防止されるので、より正確な血糖値の測定が可能になる。

【図面の簡単な説明】

【図１】第１実施形態の平面図

【図２】図１の要部拡大断面図

【図３】第２実施形態の平面図

【符号の説明】

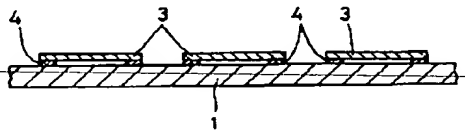
１　カード状濾紙

２　血液滴下区分

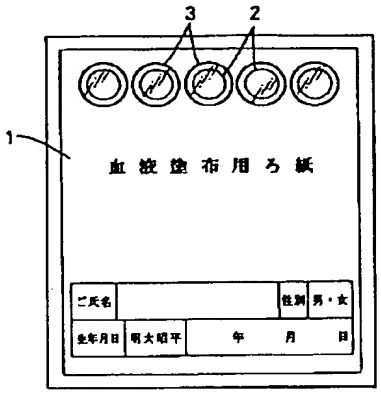
３　多孔性フィルム

４　接着剤

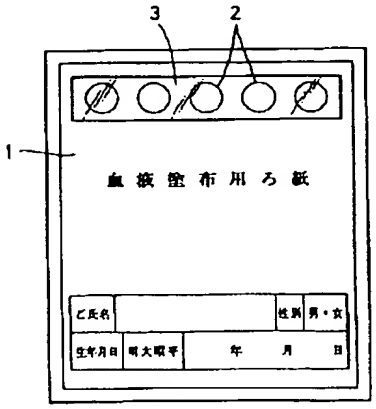
【図２】



【図１】


3
2
1
血液塗布用ろ紙
ご氏名
性別
男・女
生年月日
明大昭平
年
月
日

【図３】


3
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血液塗布用ろ紙
ご氏名
性別
男・女
生年月日
明大昭平
年
月
日

QUANTITATIVE ANALYSIS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to a quantitative analysis for determining amounts of components present in an unknown amount of specimen.

2. Related Background Art

Conventionally, a patient has been required to visit a medical institution and to have his blood, urine, or the like collected and tested, for medical treatments or diagnoses of various diseases. Usually, the test results are not available before the next medical examination or many hours. Hence, there has been a problem that such a test requires a considerably time- and- energy consuming process for both the patient and medical institution.

In order to avoid such a problem, recently, a specimen-collecting card formed, for example, of a filter paper has been proposed. For instance, JP 10-104226 A discloses a blood collecting card. Such a card has been used in the following remote clinical diagnosis system. In this remote clinical diagnosis system, a patient collects blood by himself and the blood collecting card is impregnated with the blood. This is then dried and is then mailed to a medical institution. In the medical institution that has received this, a portion impregnated with the blood is cut out from the blood collecting card and the blood is extracted to be tested with respect to various test items. When the patient visits the medical institution, medical treatments or diagnoses are conducted based on the test results.

When using such a blood collecting card, for example, since the patient himself collects blood as described above, the amount of blood with which the blood collecting card is impregnated is unknown. Hence, it has been difficult to correctly determine the amounts of components in the blood. From this viewpoint, for instance, the following methods have been proposed. In one example of the methods, filter paper capable of retaining a certain amount of blood in a certain area is used. A portion of the filter paper that has been impregnated with blood and has the certain area is cut out and thus the certain amount of blood is secured. In another example of the methods, filter paper having a certain area for retaining a certain amount of blood is used. A saturation amount of blood to be retained is supplied to the filter paper and thus the certain amount of blood is secured.

However, the aforementioned filter papers have the following problems. For instance, when using the former filer paper, the filter paper cut out is required to

have been impregnated with blood throughout, and thus selection of the portion to be cut out or a cutting operation is difficult. On the other hand, when the latter filter paper is to be impregnated with a saturation amount of blood to be retained, actually, it is necessary to supply a larger amount of blood than the saturation amount to allow the filter paper to be impregnated sufficiently with the blood. Hence, time and energy are required and thus a great burden is imposed on the patient. Furthermore, when quantitativity is intended to be improved, the manufacture of such quantitative filter papers themselves becomes very complicated and difficult, and the manufacturing cost of such filter papers increases accordingly.

Besides the methods using porous materials such as the above-mentioned filter papers, for instance, there is a method of retaining and preserving a specimen using a capillary tube with the specimen remaining in a liquid state. In this case, however, there is possibility that the specimen thus retained may be dried, or when the blood is recovered from the capillary tube using, for example, a buffer solution, the amount of the specimen contained in the recovered liquid may become unknown and thus the quantitative accuracy may be deteriorated with respect to the amounts of the components actually contained in the specimen.

SUMMARY OF THE INVENTION

The present invention at least in its preferred embodiments is intended to provide a quantitative analysis in which even when an unknown amount of specimen is used, amounts of components in the specimen can be measured with high accuracy.

In order to achieve the above-mentioned object, a quantitative analysis of the present invention is used for measuring a concentration of a component to be analyzed in a specimen. The quantitative analysis includes: measuring an amount of a component to be analyzed in a specimen; measuring an amount of a standard component present originally and homeostatically in the specimen other than the component to be analyzed; determining an amount of the specimen from the amount of the standard component thus measured and a known concentration of the standard component in the specimen; and determining a concentration of the component to be analyzed in the specimen from the amount of the specimen thus determined and the amount of the component to be analyzed thus measured. In the present invention, the standard component denotes a substance that is originally present in the specimen and has a concentration maintained to have homeostasis (to be substantially invariable), for example, in vivo.

As described above, in the quantitative analysis of the present invention, not only the amount of the component to be analyzed in the specimen but also the amount

of the standard component are measured. Thus, the concentration of the component to be analyzed in the specimen can be determined with excellent accuracy. Accordingly, the quantitativity is improved with respect to the component to be analyzed in the specimen. The standard component is a substance contained homeostatically in the specimen as described above and thus the content thereof in the specimen is known. Hence, its theoretical value (concentration) can be predetermined. Thus, for example, even in the case of a test sample containing an unknown amount of specimen, the rate of content (for example, the dilution or concentration ratio) of a specimen in the test sample can be determined from the ratio between the known concentration of the standard component and the measured amount of the standard component. Then, the concentration of the component to be analyzed actually contained in the specimen can be determined from the rate of content thus determined and the measured value of the component to be analyzed. Furthermore, according to the quantitative analysis of the present invention, it is possible to measure the amount of the component to be analyzed in the specimen without using, for example, a special porous material like one described above in order to improve the quantitativity. Thus, it also is possible to achieve cost reduction. In addition, for example, when a xenobiotica such as a dye, etc. is added as the standard material to the specimen beforehand, a problem in solubility may be caused or there is a possibility that the xenobiotica may affect the detection of the component to be analyzed. However, according to the present invention, since the aforementioned standard component is a substance originally present in the specimen, the standard component does not affect the analysis and an operation for adding such a xenobiotica is not required. Hence, the quantitative operation is simple and convenient. Consequently, the quantitative analysis of the present invention is particularly useful for various tests in clinical medical treatments, for example.

In the quantitative analysis of the present invention, preferably the specimen is retained in a porous material and is then recovered from the porous material to be analyzed. According to the quantitative analysis of the present invention, even when one of various porous materials is impregnated with an unknown amount of specimen collected by a patient himself and this is then dried and is then mailed to a medical institution as described above, the quantitative analysis of components to be analyzed in the specimen recovered as described above can be carried out easily. Hence, it is possible to save the time and energy of both the patient and medical institution, and thus the quantitative analysis of the present invention is useful for various tests in clinical medical treatments, etc., particularly for the remote clinical diagnosis system. Furthermore, the quantitative analysis of the present invention is useful, for example,

for analyzing an unknown amount of specimen as described above but is not limited thereto as long as a specimen is retained in the porous material. Besides, the quantitative analysis of the present invention also is useful in the case, for instance, where a specimen retained in a capillary tube is collected as described above.

In the quantitative analysis of the present invention, preferably, the specimen is retained in the porous material, is dried, and is then recovered.

Furthermore, it also is preferable that the porous material retaining the specimen be dipped in an extractant and the specimen be extracted from the porous material to be recovered. As described later, the amount of the extractant is not limited, but preferably, is 1 to 1000 times the porous material by volume. In addition, preferably, a ratio of the extractant to the porous material per volume is constant.

Preferably, the quantitative analysis of the present invention includes: measuring an amount of a component to be analyzed in a test sample containing an extractant and the specimen recovered from the porous material; measuring an amount of the standard component to be analyzed in the test sample; determining an amount of the specimen from the amount of the standard component thus measured and a known concentration of the standard component in the specimen; and determining a concentration of the component to be analyzed in the specimen from the amount of the specimen thus determined and the amount of the component to be analyzed in the test sample thus measured.

In the quantitative analysis of the present invention, preferably, the concentration of the component to be analyzed contained in the specimen is determined by a formula of:

$$A = Z \times (Y/X),$$

where A denotes the concentration of the component to be analyzed, Z a measured concentration value of the component to be analyzed in the test sample, X a measured concentration value of the standard component, and Y a known concentration value of the standard component in the specimen.

In the quantitative analysis of the present invention, the standard component is not limited as long as it is contained homeostatically in the specimen. Examples of the standard component include sodium ion (Na^+), chloride ion (Cl^-), potassium ion (K^+), magnesium ion (Mg^{2+}), calcium ion (Ca^{2+}), total protein (hereinafter referred to as "TP"), and albumin (hereinafter referred to as "Alb"). Among them, Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+}, and TP are preferable, Na^+, Cl^-, Mg^{2+}, Ca^{2+}, and TP are more preferable, and Na^+, Cl^-, and TP are particularly preferable.

In the quantitative analysis of the present invention, preferably, the specimen

is an aqueous liquid specimen derived from an organism. Examples of the specimen include blood, urine, saliva, lymph, a cerebrospinal fluid, and an intercellular fluid. Among them, a preferable specimen is blood or the intercellular fluid, and a more preferable specimen is blood. Any one of, for example, whole blood, blood cells, blood plasma, and blood serum can be used as the blood specimen. Preferably, the blood specimen is whole blood, blood plasma, or blood serum and more preferably, is blood plasma or blood serum. When the present invention is applied to the quantitation of such a specimen derived from an organism, for example, various diagnoses in clinical medical treatments can be conducted with high accuracy.

In the quantitative analysis of the present invention, the component to be analyzed is not limited. When the specimen is blood (blood plasma, blood serum, etc.), examples of the component to be analyzed include glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl transpeptidase (γ-GTP), creatine kinase (CPK), triglyceride (TG), amylase (Amy), HDL-cholesterol (HDL-C), and alkaline phosphatase (ALP).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the relationship between a GOT concentration obtained by correcting a GOT measured concentration with a Mg measured value and a GOT concentration in a control according to an example of the present invention.

FIG. 2 is a graph showing the relationship between a GOT concentration obtained by correcting the GOT measured concentration with a Ca measured value and the GOT concentration in the control according to the example.

FIG. 3 is a graph showing the relationship between a GOT concentration obtained by correcting the GOT measured concentration with a TP measured value and the GOT concentration in the control according to the example.

FIG. 4 is a graph showing the relationship between the GOT measured concentration and the GOT concentration in the control according to a comparative example.

FIG. 5 is a graph showing the relationship between a GPT concentration obtained by correcting a GPT measured concentration with the Mg measured value and a GPT concentration in the control according to the example.

FIG. 6 is a graph showing the relationship between a GPT concentration obtained by correcting the GPT measured concentration with the Ca measured value and the GPT concentration in the control according to the example.

FIG. 7 is a graph showing the relationship between a GPT concentration obtained by correcting the GPT measured concentration with the TP measured value

and the GPT concentration in the control according to the example.

FIG. 8 is a graph showing the relationship between the GPT measured concentration and the GPT concentration in the control according to the comparative example.

FIG. 9 is a graph showing the relationship between a γ- GTP concentration obtained by correcting a γ-GTP measured concentration with the Mg measured value and a γ-GTP concentration in the control according to the example.

FIG. 10 is a graph showing the relationship between a γ-GTP concentration obtained by correcting the γ-GTP measured concentration by the Ca measured value and the γ-GTP concentration in the control according to the example.

FIG. 11 is a graph showing the relationship between a γ- GTP concentration obtained by correcting the γ-GTP measured concentration with the TP measured value and the γ- GTP concentration in the control according to the example.

FIG. 12 is a graph showing the relationship between the γ- GTP measured concentration and the γ-GTP concentration in the control according to the comparative example.

FIG. 13 is a graph showing the relationship between a CPK concentration obtained by correcting a CPK measured concentration with the Mg measured value and a CPK concentration in the control according to the example.

FIG. 14 is a graph showing the relationship between a CPK concentration obtained by correcting the CPK measured concentration with the Ca measured value and the CPK concentration in the control according to the example.

FIG. 15 is a graph showing the relationship between a CPK concentration obtained by correcting the CPK measured concentration with the TP measured value and the CPK concentration in the control according to the example.

FIG. 16 is a graph showing the relationship between the CPK measured concentration and the CPK concentration in the control according to the comparative example.

FIG. 17 is a graph showing the relationship between a TG concentration obtained by correcting a TG measured concentration with the Mg measured value and a TG concentration in the control according to the example.

FIG. 18 is a graph showing the relationship between a TG concentration obtained by correcting the TG measured concentration with the Ca measured value and the TG concentration in the control according to the example.

FIG. 19 is a graph showing the relationship between a TG concentration obtained by correcting the TG measured concentration with the TP measured value and the TG concentration in the control according to the example.

FIG. 20 is a graph showing the relationship between the TG measured concentration and the TG concentration in the control according to the comparative example.

FIG. 21 is a graph showing the relationship between an Amy concentration obtained by correcting an Amy measured concentration with the Mg measured value and an Amy concentration in the control according to the example.

FIG. 22 is a graph showing the relationship between an Amy concentration obtained by correcting the Amy measured concentration with the Ca measured value and the Amy concentration in the control according to the example.

FIG. 23 is a graph showing the relationship between an Amy concentration obtained by correcting the Amy measured concentration with the TP measured value and the Amy concentration in the control according to the example.

FIG. 24 is a graph showing the relationship between the Amy measured concentration and the Amy concentration in the control according to the comparative example.

FIG. 25 is a graph showing the relationship between a HDL-C concentration obtained by correcting a HDL-C measured concentration with the Mg measured value and a HDL-C concentration in the control according to the example.

FIG. 26 is a graph showing the relationship between a HDL-C concentration obtained by correcting the HDL-C measured concentration with the Ca measured value and the HDL-C concentration in the control according to the example.

FIG. 27 is a graph showing the relationship between the HDL-C measured concentration and the HDL-C concentration in the control according to the comparative example.

FIG. 28 is a graph showing the relationship between an ALP concentration obtained by correcting an ALP measured concentration with the Mg measured value and an ALP concentration in the control according to the example.

FIG. 29 is a graph showing the relationship between the ALP measured concentration and the ALP concentration in the control according to the comparative example.

FIGs. 30A and 30B are a plan view and a sectional view, respectively, of a porous membrane of specimen analyzing instrument used in the example.

FIGs. 31A, 31B, and 31C are a plan view, a back side view, and a sectional view, respectively, of the specimen analyzing instrument.

FIG. 32 is a perspective view of the specimen analyzing instrument.

DETAILED DESCRIPTION OF THE INVENTION

The following description is directed to an example of a quantitative analysis according to the present invention.

Initially, a specimen is dropped on a porous material so that the porous material is impregnated with the specimen (or retains the specimen). This was dried by, for example, forced air drying or natural air drying, and then a specimen-impregnated portion of the porous material is cut out or punched out. For example, a punch can be used for the punching out. It is preferable that the place to be punched out retain many specimen-impregnated portions. According to the quantitative analysis of the present invention, however, since the amounts of components to be analyzed in an unknown amount of specimen can be determined, the quantitativity is not affected by the place to be punched out or by its area. For instance, unlike the cases of using such quantitative filter papers as described above, no problem is caused in quantitativity even when the place to be punched out includes portions that are not impregnated with the specimen.

A section obtained by the punching out or the like, for example, is cut into minute pieces and they are then put into a tube or the like. An extractant is added thereto and this is then left standing. Thus, the specimen is extracted. Subsequently, supernatant is recovered by, for example, centrifugation. The extractant is not limited as long as it can extract the specimen and does not affect the detection of the components to be analyzed in the specimen. For example, a buffer solution, a physiological salt solution, or purified water can be used as the extractant. In addition, a protein solution such as an albumin solution can be used as the extractant when it does not affect the measurement of the amounts of the components to be analyzed and standard components. Examples of the buffer solution include various buffer solutions containing, for instance, phosphoric acid, citric acid, hydrochloric acid, or acetic acid. The pH value of the buffer solution may be, for example, in the range of 3 to 9, preferably in the range of 5 to 9, and more preferably in the range of 6 to 8. It is preferable that, for example, the amount of the extractant to be added be known and can be determined according to the size of the section or the like. Specifically, the amount of the extractant to be added may be, for example, in the range of 1 to 1000 times the volume of the section, preferably in the range of 1 to 100 times, more preferably in the range of 1 to 10 times. Preferably, for instance, the amount of the extractant to be added relative to the size of the section is set to be constant, because this allows the quantitativity further to be improved. The time for the extraction process is not limited, but may be, for example, in the range of 1 to 300 minutes, preferably in the range of 1 to 180 minutes, and more preferably in the range of 10 to 60 minutes.

Next, the recovered liquid is used as a test sample, and the amounts of the components to be analyzed and the standard components in the test sample are measured. Then, the amounts of the components to be analyzed in the specimen can be determined from the values thus obtained and known concentration values (invariable concentrations) of the standard components. Specifically, the amounts of the components to be analyzed can be determined as follows.

For instance, from a measured value (concentration X) of a standard component in the test sample and a known concentration value (concentration Y) of the standard component in the specimen, a dilution ratio (Y/X) of the specimen resulted from the extraction/recovery operation is determined. Then, a measured value (concentration Z) of a component to be analyzed in the test sample (in the recovered liquid) is multiplied by the dilution ratio (Y/X), so that the concentration [Z $\times$ (Y/X)] of the component to be analyzed actually contained in the specimen can be determined. In addition, when an amount (V) of the recovered liquid is measured, the amount of the specimen [V $\times$ (X/Y)] recovered from the porous material also can be determined from the liquid amount (V) and the dilution ratio (Y/X).

The standard component is not limited as long as it is a substance with a concentration maintained homeostatically, for example, in vivo as described above. Examples of the standard component include Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+}, and TP, as described above.

The concentrations of the standard components contained in the specimen are maintained homeostatically as described above and thus can be pre-known. When being blood serum or blood plasma, the specimen includes: Na^+ with a concentration of about 134 to 146 mEq/litre (with a mean value of about 140 mEq/litre); Cl^- with a concentration of about 97 to 107 mEq/litre (with a mean value of about 102 mEq/litre); K^+ with a concentration of about 3.2 to 4.8 mEq/litre (with a mean value of about 4.0 mEq/litre); Mg^{2+} with a concentration of about 1.5 to 2.0 mEq/litre (with a mean value of about 1.8 mEq/litre); Ca^{2+} with a concentration of about 8.4 to 10.2 mEq/litre (with a mean value of about 9.3 mEq/litre); TP with a concentration of about 6.7 to 8.3 g/100 ml (with a mean value of about 7.5 g/100ml) , and Alb with a concentration of about 3.5 to 5.2 g/100ml (with a mean value of about 4.3 g/100ml).

The method of measuring the concentrations of the standard components is not limited. The concentrations of the standard components can be measured by conventionally known methods.

The concentrations of Na^+, Cl^-, and K^+ can be measured by, for example, flame photometry, a glass electrode method, a titration method, an ion selective electrode method, or a method of measuring enzyme activity of an enzyme whose

activity varies depending on the different ion concentrations (concentrations of Na^+, Cl^-, and K^+) (an enzyme activity method). Among them, the ion selective electrode method is preferable. Using the ion selective electrode method, the concentrations can be measured, for example, as follows.

In the ion selective electrode method, for instance, a standard solution is dropped on one of two ion selective electrodes and blood serum on the other. After the passage of a certain time, the potential difference between the two electrodes is measured. When the ion concentrations in the standard solution are equal to those in the blood serum, the potential difference is zero. Based on this, calibration curves each of which indicates the relationship between a potential difference and ion concentration are prepared, and thus the ion concentrations in the blood serum are determined from the calibration curves and the potential differences actually measured. For the ion selective electrode method, for example, Spot Chem SE (product name, manufactured by Arkray, Inc.) can be used as measuring equipment.

The Mg^{2+} concentration can be measured by, for example, the following xylidyl blue method. Xylidyl blue and Mg^{2+} form a chelate through the reaction expressed by the following formula:

Xylidyl Blue + Mg^{2+} → [Xylidyl Blue - Mg^{2+}] Chelate.

The xylidyl blue absorbs light with a wavelength of 620 nm but the chelate does not. Therefore, the Mg^{2+} concentration can be determined by measuring the decrease in absorbance with respect to this wavelength.

Specifically, for instance, a 3μl sample is incubated at 37 C and a 350μl coloring reagent (containing 0.13 mmol/litre xylidyl blue I (xylylazoviolet I), 0.045 mmol/litre glycol ether diamine tetraacetic acid (GEDTA), and a surfactant) is added thereto. Thus, the reaction is started. The absorbance of the reacted solution after 7.5 minutes from the start of the reaction is measured with respect to a dominant wavelength of 660nm and a sub wavelength of 700 nm. Then, the Mg^{2+} concentration is determined from the measured values and a calibration curve prepared by the measurement of a Mg standard solution with a specific concentration.

Besides, the Mg^{2+} concentration also can be measured by, for instance, the flame photometry, glass electrode method, titration method, ion selective electrode method, or enzyme activity method, as in the cases of measuring the Na^+ concentration, etc.

The Ca^{2+} concentration can be measured by, for example, a methyl xylenol blue method as described below. The methyl xylenol blue and Ca^{2+} form a chlate through the reaction expressed by the following formula:

Methyl Xylenol Blue + Ca^{2+} → [Methyl Xylenol Blue - Ca^{2+}] Chelate.

The methyl xylenol blue does not absorb light with a wavelength of 600 nm but the chelate does. Therefore, the Ca^{2+} concentration can be determined by measuring the increase in absorbance with respect to this wavelength.

Specifically, for instance, the Ca^{2+} concentration can be measured as follows. A sample of 8μl is mixed with 3.3 mol/litre monoethanolamine buffer solution (pH 12.0) of 400μl, and this is then incubated at 37 C. After five minutes, a 200μl coloring reagent (containing 0.29 mmol/litre methyl xylenol blue and 31 mmol/litre 8-quinolinol) is added thereto. Thus, the reaction is started. The absorbance of the reacted solution after five minutes from the start of the reaction is measured with respect to a dominant wavelength of 600nm and a sub wavelength of 700 nm. Then, the Ca^{2+} concentration is determined from the measured values and a calibration curve prepared by measuring the absorbance of a Ca standard solution with a specific concentration.

Besides, other methods also can be employed including, for instance, the flame photometry, glass electrode method, titration method, ion selective electrode method, or enzyme activity method, as in the cases of measuring the Na^{+} concentration and the like.

The TP concentration can be measured by, for example, a Biuret method, Lowry method, Bradford method, copper chloride method, measuring method using a refractometer, etc. Among them, the Biuret method is preferable in view of its excellent convenience.

The Alb concentration can be measured by, for example, a method using a dye such as bromocresol green (hereinafter referred to as "BCG"). Alb and BCG are bonded to each other to form a blue green dye-bound substance. Therefore, the Alb concentration can be determined by measuring the absorbance of the blue green dye-bound substance.

Specifically, for example, a 3μl sample is mixed with a 350μl BCG reagent (containing 0.18mM BCG, a 76mM succinic acid buffer solution (pH 4.25), and a nonionic surfactant). This is then allowed to react together at 37 C for 7.5 minutes. Then the absorbance of the reacted solution is measured with respect to wavelengths of 700 nm and 660 nm. Thus, the Alb concentration is determined from the measured values and a calibration curve prepared by measuring the absorbance of an Alb standard solution with a specific concentration.

As the porous material used for retaining the specimen as described above, for example, a filter paper, a glass filter, or a porous membrane made of resin can be used. Examples of the material of the porous membrane include polysulfone, polyester, nylon, cellulose nitrate, polycarbonate, and polyvinylidene fluoride. The

porous membrane may be an asymmetric porous membrane having a pore structure with its average pore size varying gradually or continuously in the thickness direction or in a direction substantially parallel to a surface of the porous membrane (an anisotropic pore-size-gradient structure). One of such porous materials may be used individually or two types of such porous materials or more may be used together. The average pore size of the porous materials is not limited as long as it allows the specimen to penetrate and to be retained, but may be, for example, in the range of 0.1 to 1000μm, preferably in the range of 0.1 to 100μm, and more preferably in the range of 5 to 50μm. The thickness of the porous material may be, for example, in the range of 10 to 1000μm, preferably in the range of 100 to 500μm, and more preferably in the range of 200 to 400μm.

In order to stably maintain the components in the specimen to be retained, the porous material may contain a stabilizer such as, for example, saccharide such as sucrose, trehalose, lactose, glucose, etc., salt such as glycine, sodium chloride, potassium chloride, etc., or a buffer such as a phosphate buffer, a citrate buffer, a Good's buffer, etc. The content of the stabilizer can be determined according to its type or the like, but may be, for example, in the range of 0.01 to 10 mg per cubic centimeter of the porous material for retaining the specimen.

When the components to be analyzed are components in blood plasma or blood serum, it is preferable, for example, to stack a blood cell separation member on the porous material. This makes it unnecessary to carry out a blood cell separation operation as a pretreatment of a blood sample in the quantitative analysis of the present invention.

The material of the blood cell separation member is not limited. For instance, a glass filter or a porous resin membrane can be used as the material. The same materials as described above can be used as the material for the porous resin membrane. The average pore size of the porous membrane is not limited as long as it allows blood cells to be separated, but may be, for example, in the range of 0.1 to 100 μm. The porous membrane may be an asymmetric porous membrane having a pore structure, for example, with its average pore size varying continuously or gradually in the thickness direction in the porous membrane.

EXAMPLE

In this example, a serum specimen retained in a porous material was recovered, amounts of standard components and various components to be analyzed in the liquid thus recovered were measured, and thus the concentrations of the components to be analyzed in the serum specimen were determined. The specimen analyzing instrument, reagents, measuring method, etc. that were used in the example

are described below.

Production of Specimen Analyzing Instrument

An asymmetric porous membrane (with a length of 35 mm, a width of 16 mm, a thickness of 320 μm, a maximum pore size of 100 μm, and a minimum pore size of 1 μm) with its average pore size varying in its thickness direction was dipped in a sucrose solution (with a concentration of 300 g/litre) and was then subjected to ultrasonication (50 kHz) for 15 minutes. This was then natural air dried. Afterward, an iron plate with a thickness of 1 mm was pressed against a portion at a location 11 mm along the length of the porous membrane with respect to one of its ends to form a groove in its width direction. Thus, the porous membrane shown in FIGs. 30A and 30B was prepared. FIG. 30A is a plan view showing the porous membrane thus processed. FIG. 30B is a sectional view taken in the direction I-I shown in the plan view. As shown in the figures, the porous membrane 10 processed as described above has a specimen supply section 11 and a specimen development section 12 that are separated by the groove formed in the width direction of the porous membrane 10. A blood cell separation section 13 is a portion between a bottom of the groove and a part of a surface of the asymmetric porous membrane corresponding to the bottom. In this porous membrane 10, the groove has a depth of 200 μm and a width of 1 mm, the specimen supply section 11 has a length of 11 mm, and the specimen development section 12 has a length of 23 mm. In the case of using this porous membrane 10, blood is dropped on a surface (the upper surface shown in FIG. 30B) of the specimen supply section 11 on the side with larger pores. While the blood moves in the thickness direction inside the specimen supply portion 11 and blood cells are separated, the blood moves in a direction(in the arrow direction shown in FIG. 30B) substantially parallel to the surface (hereinafter also referred to simply as a "surface direction"). Then, blood cells of the blood that has moved in the surface direction to reach the blood cell separation section 13 cannot pass through and are captured by the blood cell separation section 13. Consequently, only blood serum passes through the blood cell separation section 13 to be developed in the specimen development section 12.

This porous membrane 10 was set in a holder and thus the specimen analyzing instrument shown in FIGs. 31A, 31B, 31C and 32 was produced. FIG. 31A is a plan view showing this specimen analyzing instrument 1, FIG. 31B its back side view, and FIG. 31C a sectional view taken in the direction I-I shown in the plan view. FIG. 32 is its perspective view. In all the figures, the same portions are indicated with the same numerals and characters.

As shown in the figures, in this specimen analyzing instrument 1, a spacer 4

is placed around the periphery of a rectangular lower substrate 9b and a rectangular upper substrate 9a is placed thereon. Thus, the holder is formed and the porous membrane 10 is contained therein. A part of the periphery of the lower substrate 9b is not provided with the spacer 4 and thus a space between the upper substrate 9a and the lower substrate 9b is formed to serve as an air vent 6. On one side of the upper substrate 9a in its length direction, a hole 3 is formed for supplying a specimen, and the portion of the porous membrane 10 corresponding to the hole 3 is the center of the specimen supply section 11. On the inner face of the upper substrate 9a, a holding member 7 with a rectangular body is formed around the hole 3 and also has a hole communicating with the hole 3 accordingly, which serves as a specimen guide. On the inner face of the lower substrate 9b, a protruding supporter 8 is formed to protrude in the width direction at a location slightly closer to the center of the lower substrate 9b in the transverse direction than its portion corresponding to the hole 3 to the center. The protruding supporter 8 lifts a part of the blood cell separation section 13 of the porous membrane 10, whereby a gap is formed between the inner face of the lower substrate 9b and the porous membrane 10. Two holding members 5 with rectangular bodies formed on the inner face of the upper substrate 9a fix both ends of the development section12 of the porous membrane 10 along its longitudinal direction to the inner wall of the lower substrate 9b.

Blood-Specimen Impregnation and Blood-Serum Recovery Methods

Through the hole 3 for specimen supply of the specimen analyzing instrument 1, about 100 μl whole blood of a healthy subject was dropped on the porous membrane 10. As described above, blood cells in the whole blood were separated in the blood-cell separation section 13 and blood serum was allowed to develop sufficiently in the development section 12. This was then natural-air-dried for 24 hours. Afterward, the porous membrane 10 was removed from the specimen analyzing instrument 1 and the development section 12 was cut out. The cut portion further was cut in a strip shape and thus cut pieces were obtained. The cut pieces were put into a test tube, to which a 150 μl PBS solution (phosphate-buffered saline, with pH 7.4, the same is true for the following description) was added as an extractant. This was left standing at room temperature for 20 minutes and was then subjected to centrifugation. As a result, supernatant was obtained and was used as a test sample. In the same manner as described above, a total of 11 test samples were prepared with whole blood of 11 healthy subjects, respectively. Then, as shown in the below, the respective test samples were subjected to the measurement of amounts of various standard components and various components to be analyzed. In this case, amounts of Ca^{2+}, Mg^{2+}, and TP as the standard components were measured.

The amounts of the respective standard components and various components to be analyzed were measured using the following commercially available measuring kits according to their application methods with an autoanalyzer (BM-8 manufactured by Nippon Electronic Co., Ltd.). In various measurements, purified water was used as a blank.

Measuring Kits for Standard Components

1. Ca^{2+}

Product Name : Calcium E-HA Test Wako (Wako Pure Chemical Industries, Ltd.) (a methyl xylenol blue method)

2. Mg^{2+}

Product Name : Magnesium - HRII (Wako Pure Chemical Industries, Ltd.) (a xylidyl blue method)

3. TP

Product Name : TP II-HA Wako (Wako Pure Chemical Industries, Ltd.)

Measuring Kits for Components to be analyzed

1. Glutamic - Oxaloacetic Transaminase (GOT)

Product Name : Transaminase HR-II (GOT-7070: Wako Pure Chemical Industries, Ltd.)

2. Glutamic - Pyruvic Transaminase (GPT)

Product Name : Transaminase HR-II (GPT-7070: Wako Pure Chemical Industries, Ltd.)

3. γ- Glutamyl Transpeptidase (γ-GTP)

Product Name : γ-GTP J-HA Test Wako (Wako Pure Chemical Industries, Ltd.)

4. Creatine Kinase (CPK)

Product Name : CK E-HA Test Wako (Wako Pure Chemical Industries, Ltd.)

5. Triglyceride (TG)

Product Name : Triglyceride E-HA Test Wako (Wako Pure Chemical Industries, Ltd.)

6. Amylase (Amy)

Product Name : Amy II-HA Test Wako (Wako Pure Chemical Industries, Ltd.)

7. HDL-Cholesterol (HDL-C)

Product Name : Choletest HDL (Daiichi Pur Chemicals Co., Ltd.)

8. Alkaline Phosphatase (ALP)

Product Name : ALP II-HA Test Wako (7150 : Wako Pure Chemical Industries, Ltd.)

As an example, measured values of the various components to be analyzed and various standard components in the test samples were substituted in the following

formula (1) and thus the amounts of the various components to be analyzed in the blood serum specimens were determined.

Concentration of a component to be analyzed, contained in a specimen = $Z \times (Y/X)$ .....(1)

Z: Measured value (concentration) of a component to be analyzed in a test sample

X: Measured value (concentration) of a standard component in the test sample

Y: Known theoretical value (concentration) of the standard component in a specimen

Known theoretical values (concentrations) of the standard components in the blood serum specimens are as follows: the Mg^{2+} concentration is 1.8 mEq/litre, the Ca^{2+} concentration 9.3 mEq/litre, and the TP concentration 7.5 g/100ml. As a comparative example, the corrections with the standard components expressed by the formula (1) were not carried out. As a control, whole blood of the same healthy subjects was subjected to centrifugation, blood serum thus obtained was used, and amounts of various components to be analyzed in the blood serum were measured by the measuring method as described above.

With respect to the measurement results of the example, graphs were prepared, each of which shows a concentration of a component to be analyzed in the control (indicated in the x-axis) and a concentration of the component to be analyzed after a correction (indicated in the y-axis). Besides, with respect to the measurement results of the comparative example, graphs were prepared, each of which shows a concentration of a component to be analyzed in the control (indicated in the x-axis) and a concentration of the component to be analyzed subjected to no correction (indicated in the y-axis). Thus, correlation coefficients and slopes of the formulae expressing the respective relationships were determined. These results are shown in Tables 1 and 2 below.

Table 1

Correlation Coefficient R^2

	Example			Comparative Example
	Correction with Mg	Correction with Ca	Correction with TP	
GOT	0.926	0.876	0.831	0.556
GPT	0.979	0.974	0.929	0.538
γ-GTP	0.987	0.984	0.985	0.794
CPK	0.973	0.919	0.859	0.629

TG	0.982	0.982	0.969	0.0021
Amy	0.801	0.698	0.734	0.499
HDL-C	0.805	0.782	-	0.520
ALP	0.766	-	-	0.556

Table 2

Slopes in Correlation Equation

	Example			Comparative Example
	Correction with Mg	Correction with Ca	Correction with TP	
GOT	0.449 (0.996)	0.467 (1.04)	0.598 (0.998)	0.119 (1.003)
GPT	0.362 (0.905)	0.395 (0.988)	0.618 (1.16)	0.0796 (0.986)
γ-GTP	0.543 (0.988)	0.558 (1.02)	0.767 (1.05)	0.114 (0.935)
CPK	0.495 (0.989)	0.521 (1.04)	0.720 (1.08)	0.125 (0.988)
TG	0.584 (0.975)	0.619 (1.03)	0.813 (1.02)	0.126 (0.904)
Amy	0.606 (1.01)	0.642 (1.07)	0.789 (0.987)	0.150 (0.992)
HDL-C	0.460 (0.919)	0.474 (0.948)	-	0.106 (1.01)
ALP	0.543 (0.988)	-	-	0.114 (0.997)

* The values indicated in the parentheses indicate slopes after multiplication by the coefficients.

FIGs. 1 to 29 show graphs obtained by plotting of concentration values obtained by multiplying the concentrations of the components to be analyzed after the corrections in the example and the concentrations of the components to be analyzed subjected to no correction in the comparative example, by the coefficients mentioned in Table 3 below. The coefficients serve to set the recovery of each component to be analyzed from the porous membrane to be 100%. These coefficients can be calculated from the recovery of the respective components to be analyzed and the respective standard components from the porous membrane. The recovery is determined as follows: for example, under the same conditions as described above, the porous membrane is impregnated with a known amount of blood serum specimen containing a component to be analyzed with a known concentration, then the blood serum specimen is recovered from the porous membrane, and the component to be analyzed and the standard component in the recovered liquid are measured.

Table 3

Coefficients for Various Components

	Standard Component		
Component to be analyzed	Mg^{2+}	Ca^{2+}	TP
GOT	2.22	2.22	1.67
GPT	2.50	2.05	1.88
γ-GTP	1.82	1.82	1.36
CPK	2.00	2.00	1.50
TG	1.67	1.67	1.25
Amy	1.67	1.67	1.25
HDL-C	2.00	2.00	1.50
ALP	1.82	1.82	1.36

In FIGs. 1 to 29, each of graphs (A), (B), and (C) shows the relationship between a concentration of a component to be analyzed in the control (indicated in the x-axis) and a concentration of the component to be analyzed after a correction (indicated in the y-axis) in the example. The graphs (A), (B), and (C) show results of the corrections using Mg^{2+}, Ca^{2+}, and TP, respectively. Each of graphs (D) shows the relationship between a concentration of a component to be analyzed in the control (indicated in the x-axis) and a concentration of the component to be analyzed subjected to no correction using the standard components (indicated in the y-axis) in the comparative example. The formulae in the figures indicate the relationships and R^2 denotes a correlation coefficient. FIGs. 1 to 4 show results of GOT analysis, FIGs. 5 to 8 results of GPT analysis, FIGs. 9 to 12 results of γ-GTP analysis, FIGs. 13 to 16 results of CPK analysis, FIGs. 17 to 20 results of TG analysis, FIGs. 21 to 24 results of Amy analysis, FIGs. 25 to 27 results of HDL-C analysis, and FIGs. 28 and 29 results of ALP analysis. The slopes of the correlation equations obtained from these graphs are shown in Table 2 above.

As shown in Table 1 and in the respective figures, the correlation coefficients in the example were higher than those in the comparative example, and there was a high correlation between the concentrations of the components to be analyzed in the example and those in the control. Thus, according to the quantitative analysis of the present invention, the dilution ratio of a specimen in a test sample can be determined through the measurement of amounts of standard components, and thereby the concentrations of the components to be analyzed in the specimen can be measured further correctly. The multiplication by the coefficients is carried out to suppose the recovery is 100%. Hence, the multiplication does not improve the correlation coefficient indicating the measurement accuracy, but the slopes of the correlation equations come closer to "1" in both the example and comparative example, as shown

in Table 2 and in the respective figures.

As described above, the quantitative analysis of the present invention allows an amount of a specimen in a test sample to be determined and thus improves the quantitativity with respect to components to be analyzed in the specimen. Such a quantitative analysis of the present invention is useful for diagnosis in clinical medical practice or the like.

The invention may be embodied in other forms without departing from the spirit or essential characteristics thereof. The embodiments disclosed in this application are to be considered in all respects as illustrative and not limiting. The scope of the invention is indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A quantitative analysis for measuring a concentration of a component to be analyzed in a specimen, comprising:

measuring an amount of a component to be analyzed in a specimen;

measuring an amount of a standard component present originally and homeostatically in the specimen other than the component to be analyzed;

determining an amount of the specimen from the amount of the standard component thus measured and a known concentration of the standard component in the specimen; and

determining a concentration of the component to be analyzed in the specimen from the amount of the specimen thus determined and the amount of the component to be analyzed thus measured.

2. The quantitative analysis according to claim 1, wherein the standard component is at least one selected from the group consisting of sodium ion, chloride ion, potassium ion, magnesium ion, calcium ion, total protein, and albumin.

3. The quantitative analysis according to claim 2, wherein the standard component is at least one selected from the group consisting of magnesium ion, calcium ion, and total protein.

4. The quantitative analysis according to claim 1, wherein the specimen is an aqueous liquid specimen derived from an organism.

5. The quantitative analysis according to claim 4, wherein the aqueous liquid specimen is at least one selected from the group consisting of blood, urine, saliva, lymph, a cerebrospinal fluid, and an intercellular fluid.

6. The quantitative analysis according to claim 5, wherein the aqueous liquid specimen is blood.

7. The quantitative analysis according to claim 1, wherein the component to be analyzed is at least one selected from the group consisting of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl transpeptidase (γ-GTP), creatine kinase (CPK), triglyceride (TG), amylase (Amy), HDL-cholesterol (HDL-C), and alkaline phosphatase (ALP).

8. The quantitative analysis according to claim 1, wherein the specimen is retained in a porous material and is then recovered from the porous material for analysis.

9. The quantitative analysis according to claim 8, wherein the specimen is retained in the porous material, is dried, and is then recovered.

10. The quantitative analysis according to claim 8, wherein the porous material retaining the specimen is dipped in an extractant and the specimen is extracted from the porous material to be recovered.

11. The quantitative analysis according to claim 10, wherein the extractant is at least one selected from the group consisting of a buffer solution, a physiological salt solution, and purified water.

12. The quantitative analysis according to claim 10, wherein an amount of the extractant is 1 to 1000 times the porous material by volume.

13. The quantitative analysis according to claim 10, wherein a ratio of the extractant to the porous material per volume is constant.

14. The quantitative analysis according to claim 10, wherein the quantitative analysis comprises:

measuring an amount of a component to be analyzed in a test sample containing an extractant and a specimen recovered from the porous material;

measuring an amount of the standard component to be analyzed in the test sample;

determining an amount of the specimen from the amount of the standard component thus measured and a known concentration of the standard component in the specimen; and

determining a concentration of the component to be analyzed in the specimen from the amount of the specimen thus determined and the amount of the component to be analyzed in the test sample thus measured.

15. The quantitative analysis according to claim 14, wherein the concentration of the component to be analyzed contained in the specimen is determined by a formula:

$$A = Z \times (Y/X),$$

where A denotes the concentration of the component to be analyzed, Z denotes a measured concentration value of the component to be analyzed in the test sample, X denotes a measured concentration value of the standard component, and Y denotes a known concentration value of the standard component in the specimen.

16. The quantitative analysis according to claim 1, wherein the specimen is blood serum or blood plasma, and known concentration values of the standard components contained in the specimen comprise 134 to 146 mEq/litre of sodium ion, 97 to 107 mEq/litre of chloride ion, 3.2 to 4.8 mEq/litre of potassium ion, 1.5 to 2.0 mEq/litre of magnesium ion, 8.4 to 10.2 mEq/litre of calcium ion, 6.7 to 8.3 g/100 ml of total protein, and 3.5 to 5.2 g/100ml of albumin.

17. The quantitative analysis according to claim 1, wherein the specimen is blood serum, the standard component is magnesium ion, and the magnesium ion in the blood serum has a known concentration of 1.8 mEq/litre.

18. The quantitative analysis according to claim 1, wherein the specimen is blood serum, the standard component is calcium ion, and the calcium ion in the blood serum has a known concentration of 9.3 mEq/litre.

19. The quantitative analysis according to claim 1, wherein the specimen is blood serum, the standard component is total protein, and the total protein in the blood serum has a known concentration of 7.5 g/100ml.

ABSTRACT OF THE DISCLOSURE

Amounts of components in a specimen can be analyzed with excellent quantitativity. The analysis includes: measuring an amount of a component to be analyzed in a specimen; measuring an amount of a standard component present originally and homeostatically in the specimen other than the component to be analyzed; determining the amount of the specimen from the amount of the standard component thus measured and a known concentration of the standard component in the specimen; and determining a concentration of the component to be analyzed in the specimen from the amount of the specimen thus determined and the amount of the component to be analyzed thus measured. The quantitative analysis of the present invention allows a component to be analyzed to be measured with high quantitativity as shown in FIG. 1.